American Journal of Medical Genetics 63:161–166 (1996)

Deficient Expression of the Small Proteoglycan Decorin in a Case of Severe/Lethal Osteogenesis Imperfecta

Katharine M. Dyne, Maurizia Valli, Antonella Forlino, Monica Mottes, Hans Kresse, and Giuseppe Cetta

Department of Biochemistry, University of Pavia, Pavia (K.M.D., M.V., A.F., G.C.), Istituto di Biologia e Genetica, University of Verona, Verona, Italy (M.M.); and Institute of Physiological Chemistry and Pathobiochemistry, University of Münster, Münster, Germany (H.K.)

In osteogenesis imperfecta (OI) the effects of mutations in type I collagen genes generally reflect their nature and localization. Unrelated individuals sharing identical mutations present, in general, similar clinical phenotypes. However, in some such cases the clinical phenotype differs. This variable clinical expression could be the result of abnormalities in other connective tissue proteins. Since decorin is a component of connective tissue, binds to type I collagen fibrils and plays a role in matrix assembly, we studied decorin production in skin fibroblasts from OI patients.

Cultured fibroblasts from one patient with extremely severe osteogenesis imperfecta (classified as type II/III) who has an α1(I)gly415ser mutation were found to secrete barely detectable amounts of decorin into culture medium. Western blotting using antibodies raised against decorin confirmed the reduction of the decorin core protein and Northern blot analysis showed decorin mRNA levels below the limit of detection. Cells from a patient, with a less severe phenotype, bearing a mutation in the same position of the triple helix (α1(I)gly415) expressed decorin normally. The different clinical phenotypes could be due to the differing genetic backgrounds of the patients so it is tempting to conclude that in our most severely affected patient the absence of decorin aggravates the clinical phenotype.

Received for publication January 2, 1996; revision received January 11, 1996.

Address reprint requests to Dr. Katharine M. Dyne, Department of Biochemistry, University of Pavia, Via Taramelli 3B, 27100 Pavia, Italy.

Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

KEY WORDS: decorin, osteogenesis imperfecta, type I collagen

INTRODUCTION

Mutations in the type I collagen genes causing altered type I collagen secretion and function are responsible for osteogenesis imperfecta (OI) [Byers, 1993]. The exact mechanism by which mutations in the primary structure of collagen cause alterations in the connective tissues and result in the different OI phenotypes is still not fully understood. Generally, the effects of the mutations reflect their nature and localization. Most mutations are unique to each individual (and his family) but there are increasingly more reports of unrelated OI patients who share identical mutations and have the same phenotype. However, it is puzzling that some unrelated individuals bear mutations in the same position along the collagen triple helix and present different clinical phenotypes. This variable expression could be the result of abnormalities in other connective tissue proteins.

Molecules in the extracellular matrix (ECM) interact with each other and with their specific cell surface receptors and exert an influence on gene expression, so it is likely that altered interactions between different matrix molecules play a part in causing connective tissue abnormalities. Mutations in type I procollagen genes could affect expression and metabolism of other connective tissue proteins that are functionally involved with collagen fibrils. Decorin could be a candidate, because of its important role in matrix organization and assembly and its ability to bind to type I collagen.

Decorin belongs to a family of structurally related small interstitial proteoglycans with wide tissue distribution [Bianco et al., 1990] and it has the ability to bind to different extracellular matrix constituents and growth factors [Kresse et al., 1993]. It binds specifically via its core protein to the surface of fibrillar collagens [Scott, 1988; Fleischmajer et al., 1991] and it appears to influence in vitro fibrillogenesis of types I and II colla-

gens [Vogel et al., 1984] and has an affinity for type VI collagen [Bidanset et al., 1992] and type XIV collagen [Font et al., 1993]. Decorin also interacts with fibronectin [Schmidt et al., 1991], influences cell adhesion [Winnemöller et al., 1991], and appears to have an important biological role in regulation of cell proliferation through its ability to bind transforming growth *factor-β* [Yamaguchi et al., 1990] and may have a role in development of cancer [Santra et al., 1995].

We have previously reported on a case of severe/lethal OI due to a α1(I)Gly415Ser mutation [Mottes et al., 1993]. Two other unrelated cases of OI, one due to an identical mutation [Bateman et al., 1992] and another with a Gly415Cys substitution [Nicholls et al., 1991], both resulting in moderate OI type III/IV, have also been described. The mutation at position 415 falls in the d band of collagen fibrils which is thought to be involved in interaction with proteoglycans [Scott, 1988]. For this reason we investigated expression of decorin in skin fibroblasts derived from patients with mutations in this position along the triple helix. We also extended our studies to other OI cell lines bearing other mutations in one or other collagen genes COL1A1 or COL1A2 scattered along the length of the triple helix.

MATERIALS AND METHODS

Fibroblast Cell Lines

Skin fibroblasts from our patient 1 (α1(I)Gly415Ser) and patient 2 (α1(I)Gly415Cys) and 12 other patients with different types of osteogenesis imperfecta and varying defects in type I collagen were used in this study. The exact mutation was known in most cases (Table I). Control and OI cells were grown in Dulbecco's Modified Eagles medium (DMEM) containing 10% neonatal calf serum and used between passages 5–16 for all experiments.

Metabolic Labelling of Cells in Culture

Cells were plated on 35 mm Petri dishes (25×10^4 cells/dish) in DMEM containing serum and left to grow to near confluence. Before labelling, cells were incubated in serum-free DMEM containing ascorbate(100 μg/ml) for 3 h. For labelling, medium was replaced with serum-free DMEM containing ascorbate and 50 μCi/ml carrier-free [^{35}S]sulfate (NEN) and/or 20 μCi/ml [^{3}H]leucine (Amersham) for 24 h.

Isolation and Analysis of Proteoglycans

After labelling medium fractions were applied to a 1 ml column of DEAE-Sephacel equilibrated in urea buffer (7 M urea, 10 mM Tris/HCl, pH 8.0, containing protease inhibitors (PI: 0.1 M ε-aminohexanoic acid, 10 mM EDTA disodium salt, 5 mM benzamidine hydrochloride, 10 mM N-ethylmaleimide and 1 mM phenylmethylsulfonyl fluoride), and 0.5% Triton X-100. Three milliter urea buffers were applied to wash the column and then 3 ml each of this buffer containing 0.1 M NaCl and 0.2 M NaCl, respectively, were passed through the column. Proteoglycans were eluted in 1.5 ml 4 M guanidine hydrochloride/0.05 M sodium acetate, 0.5% Triton X-100 and PI, pH 5.8, precipitated with 9 volumes 95% ethanol, vacuum-dried, and subjected to SDS-PAGE on a linear 4–15% gradient prior to fluorography using En3Hance (NEN). Aliquots of the proteoglycan fractions were taken up in 100 mM Tris/HCl, 30 mM sodium acetate, pH 8.0, containing PI, treated with protease-free chondroitin ABC lyase (Seikagaku) to remove the GAG moiety and electrophoresed on 8% gels. Immunoblotting was performed using antibodies raised against human decorin [Glössl et al., 1984].

RNA Extraction and Northern Blot Analysis

Total RNA from confluent fibroblast cell layers was isolated using RNA-fast (Molecular Systems). For Northern analysis 10 μg total RNA was electrophoresed on 1.5% formaldehyde-agarose gels and transferred onto a Hybond™-N nylon membrane (Amersham). cDNA probes used were Plasmid P2 and P16, kind gifts from Dr. Larry Fisher: plasmid P2 contains the cDNA encoding for human bone decorin [Fisher et al., 1989] and P16 contains the complete coding sequence of human bone biglycan [Fisher et al., 1989]. Full length pro α1(I) cDNA probe was a kind gift

TABLE I. Skin Fibroblasts From Patients With Osteogenesis Imperfecta: The Clinical Phenotype, Mutation in Type I Collagen and Effect on Collagen Secretion in the Cases Investigated

Cell strain	Phenotype	Mutation	Collagen secretion	Reference
Patient 1	OI II/III	α1(I)Gly415Ser	Intracellular accumulation	Mottes et al., 1993
Patient 2	OI III/IV	α1(I)Gly415Cys	Intracellular accumulation	Nicholls et al., 1991
276	OI I	α1(I)Gly85Val	Normal	Valli et al., 1993c
291	OI I	α2(I) Δ cx 20	Instability of mutant collagen	Mottes et al., 1994
156	OI I	α1(I)Gly901Ser	Intracellular accumulation	Mottes et al., 1992
160	OI I/IV	α1(I)Gly178Cys	Normal	Valli et al., 1991
165	OI III	α1(I)Gly922Ser	Intracellular accumulation	D'Amato et al., 1993
307	OI III	a1(1)Gly589Ser	Normal	Forlino et al., 1994
325	OI III	a1(I)Gly589Ser	Normal	Forlino et al., 1994
332	OI III	α2(I)Gly586Val	Normal	Forlino et al., 1994
242	II	α1(I)Gly910Ala	Intracellular accumulation	Valli et al., 1993a
383	II	α1(I)Gly994Asp	Intracellular accumulation	Mottes, unpublished
405	II	Unknown	Intracellular accumulation	Valli, unpublished
440	II	α2(I)Gly319Val	Intracellular accumulation	Mottes, unpublished

from Dr. F. Ramirez, Mount Sinai Hospital, New York. The β-actin cDNA probe was as described [Ponte et al., 1983]. cDNA probes were radioactively labelled to a specific activity of 5–10 × 10^8 dpm/μg with [^{32}P]-dCTP (Amersham) using Megaprime™ DNA Labelling System (Amersham). Prehybridization and hybridization of the filters were performed with Rapid-Hyb Buffer (Amersham). The filters were exposed on Hyperfilm Autoradiography Film with intensifying screen for 1–3 days. Quantification of Northern blots was by densitometric scanning of fluorograms and correlation with either β-actin mRNA or rRNA.

RESULTS

Protein Studies on Decorin

Patient 1 is heterozygous for a point mutation resulting in the substitution of glycine 415 for serine in the α1 chain of type I collagen. He inherited the mutation from his unaffected father who is mosaic in both his germline and somatic tissues for the mutation. The clinical, genetic, and biochemical characteristics were described previously [Mottes et al., 1993; Valli et al., 1993b]. Briefly, the propositus was the product of a second pregnancy to healthy, unrelated parents. On ultrasonography at 24 weeks gestation he presented anomalies compatible with a diagnosis of extremely severe OI. A previous pregnancy had resulted in an infant who died of respiratory failure within 1 h of birth, with clinical and radiological features of very severe OI, falling between type II and type III Sillence's classification. Another subsequent pregnancy was terminated after the same diagnosis was made at 20 weeks gestation. A skin biopsy was available from propositus 1 only. Studies showed that type I collagen was poorly secreted and mutant molecules were deposited in the cell matrix. Patient 2, with a point mutation in the same position but different substituting aminoacid (Gly415Cys), is a patient in his fifties with moderately severe OI type III/IV [Nicholls et al., 1991]. His fibroblasts presented similar biochemical findings as those of patient 1.

Skin fibroblasts were labelled with [^{35}]S] sulfate and [^{3}H] leucine and proteoglycans isolated from culture medium as described. We found that the quantity of [^{35}S] sulfate-labelled proteoglycans in the culture medium of patient 1 was very low (<70%), when referred to total protein synthesis, measured as [^{3}H] leucine incorporation. On the contrary, radiosulfate incorporation by fibroblasts from patient 2 and some other patients was somewhat variable but not significantly different from that of controls. For all cases, apart from propositus 1, on SDS/PAGE the pattern of secreted proteoglycans was essentially normal, i.e., intact decorin, the predominant proteoglycan from medium, migrated as a diffuse band to a position corresponding to proteins of about 97–120 kDa, and biglycan, present in smaller amounts, migrated like proteins of 150–200 kDa. There was some variability in the amount of mature decorin between cell lines but we found that these differences were inconsistent between experiments (Fig. 1a,b). In the case of patient 1 medium decorin was barely detectable and this finding was consistent in 6 independent experiments (Fig. 1b).

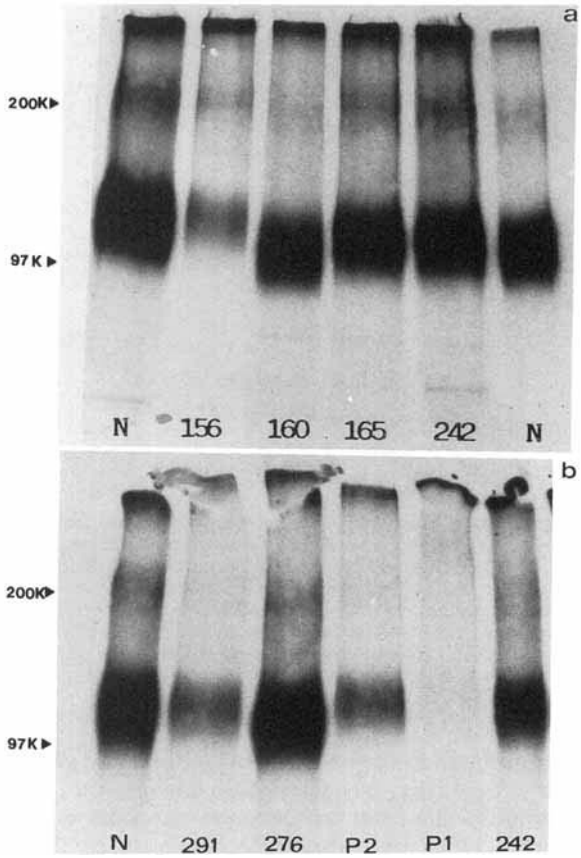

Fig. 1. SDS-PAGE (4–15% gradient) of ^{35}S-labelled proteoglycans in medium of normal and OI fibroblast cultures. **a:** Control (N) and most OI cells secreted predominantly decorin with lower amounts of biglycan. **b:** Cells from patient 1 (P1) secreted barely detectable amounts of decorin, patient 2 (P2).

After digestion with chondroitinase ABC, core proteins were analysed by SDS/PAGE and immunoblotting using antiserum to human decorin. As expected, decorin core proteins migrated as a doublet (a major band with a molecular weight just above 46 kDa and a minor band which migrated a little faster). In all OI and normal cell strains, except the one from patient 1, there were no obvious differences in quantity and electrophoretic mobility of decorin core proteins. However, the reduction in decorin was confirmed at the level of the core proteins in patient 1 in several independent experiments (Fig. 2) and subsequent Northern blot analysis showed that decorin mRNA was below the limit of detectability (Fig. 3). For patient 2 (Fig. 3) and the other OI cells (not all shown), Northern blot analysis confirmed that decorin expression by these OI cells was not particularly different from control cells. After correlation with β-actin mRNA, biglycan and type I collagen α1(I) mRNA levels were normal for all cell lines including patient 1 (not shown). Northern blot analyses were performed three times.

To test the possibility that patient 1 could have inherited the defect in decorin we thought it worthwhile

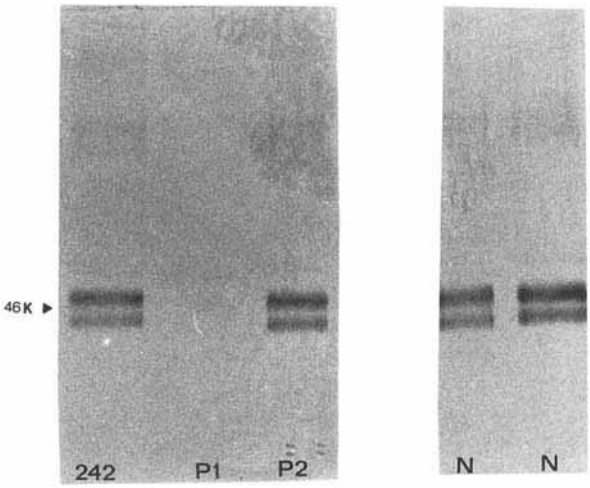

Fig. 2. Western blot analysis of decorin core proteins from medium of normal and OI fibroblast cultures. Proteoglycans were isolated from media as described and digested with chondrointin ABC lyase. Core proteins were then subject to SDS/PAGE, transferred to nitrocellulose membranes, and probed with antibody to human decorin core protein. Core proteins were not detectable in the case of patient 1 (P1). Control (N), patient 2 (P2) and another OI patient (OI242).

investigating decorin production by fibroblasts from his parents. Secretion of polypeptide decorin into culture medium by cells from the parents was more or less as for control cells. Decorin mRNA levels were within the range of controls for the patient's father. In the case of the patient's mother the signal for decorin mRNA appears weaker but total RNA was lower than that of controls (Fig. 4). This blot was performed only once.

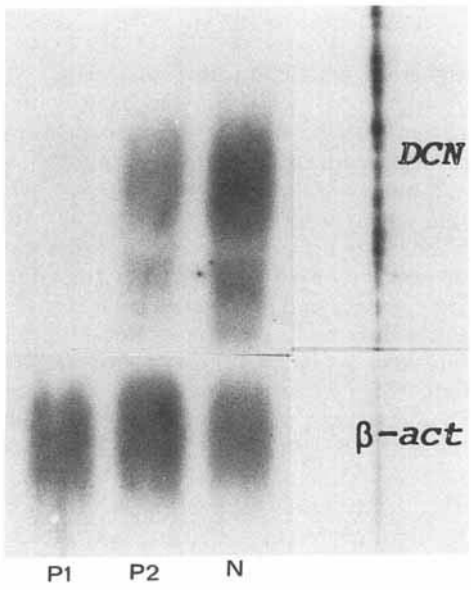

Fig. 3. Northern blot analysis of decorin (DCN) mRNA in fibroblasts from patients 1 and 2 (P1, P2) and a normal subject (N). To check the quantity of RNA the same RNA filter was also hybridized with the cDNA probe specific for β-actin. Decorin mRNA levels were found to be barely detectable in the case of patient 1 (P1).

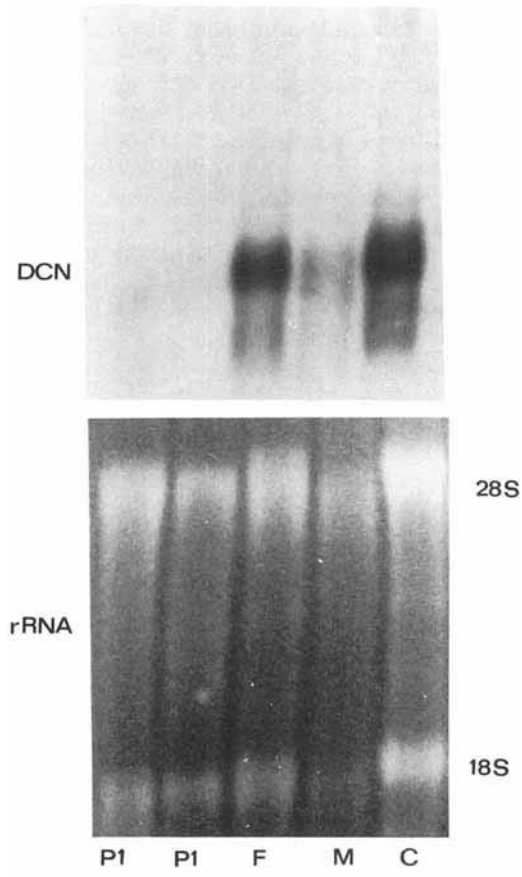

Fig. 4. Northern blot analysis of decorin mRNA in fibroblasts from patient 1 (P1), his father (F), mother (M) and a control (C). The (^{32}P) cDNA-mRNA hybrids were visualized by autoradiography (upper panel). Ethidium bromide staining of RNA prior to transfer is shown in the lower panel.

DISCUSSION

We report deficient expression of decorin in a case of severe/lethal OI: fibroblasts from the propositus secreted only trace amounts of the proteoglycan decorin into culture medium (the major secretory proteoglycan in human skin fibroblast cell cultures), and levels of decorin mRNA were below the limit of detection. Since fibroblasts from this propositus showed normal levels of type I collagen and biglycan mRNA, the reduction in decorin was not simply the result of a general reduction in expression of ECM proteins. However, abnormal expression of decorin was not found in cells from patient 2 bearing a mutation in the same position or cells from the other OI patients. The finding of abnormal decorin expression in patient 1 in particular is puzzling. Expression of decorin is not linked to patient's age or passage number of cultured skin fibroblasts in agreement with previous findings [Vogel and Clark, 1989; Beavan et al., 1993; Pulkkinen et al., 1990]. Low decorin expression does not appear to be the result of poor secretion secondary to intracellular retention of abnormal type I collagen either as shown by Vogel and Clark [1989] and confirmed by our results.

To date, no mutation in the decorin gene has been described in a connective tissue disorder [Sztrolovics

et al., 1994], although abnormally low expression of decorin has been reported in fibroblasts from some cases of neonatal Marfan syndrome [Pulkkinen et al., 1990; Raghunath et al., 1993], and progeria [Beavan et al., 1993] and a non-glycanated form of decorin due to galactosyl transferase I deficiency has been reported in a patient with a progeroid-like syndrome [Quentin et al., 1990]. Marfan syndrome is caused by mutations in the fibrillin gene while the primary defect in progeria is still unknown.

There are few studies on the effect that altered collagen function in OI has on other matrix components: a general decrease in small proteoglycan synthesis by bone cells from OI patients has been reported [Fedarko et al., 1992], although another study of the same group showed that in human OI bone decorin levels were not significantly altered [Vetter et al., 1991].

The rate of decorin biosynthesis was somewhat variable in the different cell strains we studied, possibly being due to either some variations in core protein expression or to variations in post-translational events. However, an almost complete absence of decorin is confined to patient 1. Decorin expression by skin fibroblasts with a type I collagen mutation in the same position (patient 2) was normal as was decorin expression in other OI cell lines with mutations falling both in the gap and the overlap zone of the triple helix. Unfortunately, we did not have skin fibroblasts from the patient of Bateman et al. [1992] bearing the same mutation to study decorin expression. The group of 14 OI patients we studied is not large, but abnormal decorin expression does not appear to be a general finding in OI fibroblasts. Fedarko et al. [1992] measured the radiosulfate incorporation in OI osteoblasts and found a general reduction of decorin in all their patients regardless of clinical phenotype: however, they did not report any data on chondroitinase ABC treated decorin or decorin mRNA levels. It is possible that the abnormal decorin expression in our patient's cells is a secondary phenomenon although one might speculate that the proband's cells bear another defect that impairs decorin transcription. In view of the clinical phenotypes (both moderately severe, OI III/IV) of patient 2 and the other individual reported by Bateman et al. [1992], bearing an identical mutation, our patient 1's clinical outcome, which was the severest, was surprising. A hypothesis for this finding is the differing genetic backgrounds of the patients, so it is tempting to conclude that in patient 1 the absence of decorin makes the clinical phenotype more severe.

Since decorin is implicated as having an important role in control of cell growth and proliferation, studies on the growth and proliferation of the patients' decorin deficient cells in the presence and absence of growth factors are currently in progress.

ACKNOWLEDGMENTS

We thank Dr. Alan Nicholls (Dermatology Research Centre, UK) for providing us with fibroblasts from patient 2, Angelo Gallanti for culture of fibroblasts, and Prof. PF Pignatti (Institute of Biologia e Genetica, University of Verona, Italy) for providing laboratory facilities for performing Northern blot analyses. This work was supported by MURST 40% and 60%, CNR Target Project "Biotecnologia e Biostrumentazione," and Regione Lombardia area 525, progetto 723.

REFERENCES

Bateman JF, Moeller I, Hannagan M, Chan D, Cole WG (1992): Characterization of three osteogenesis imperfecta collagen α1(I) glycine to serine mutations demonstrating a position dependent gradient of phenotypic severity. Biochem J 288:131–135.

Beavan LA, Quentin-Hoffman E, Schönherr E, Snigula F, Leroy JG, Kresse H (1993): Deficient expression of decorin in infantile progeroid patients. J Biol Chem 268:9856–9862.

Bianco P, Fisher LW, Young MF, Termine JD, Gehron Robey PJ (1990): Expression and localization of the two small proteoglycans biglycan and decorin in developing skeletal and non-skeletal tissues. J Histochem Cytochem 38:1549–1563.

Bidanset DJ, Guidry C, Rosenberg LC, Choi HU, Timpl R, Hook M (1992): Binding of the proteoglycan decorin to collagen type VI. J Biol Chem 267:5250–5256.

Byers PH (1993): Osteogenesis Imperfecta. In Royce PM and Steinmann B (eds): "Connective Tissue and Its Heritable Disorders: Medical and Genetic Aspects." New York: Wiley-Liss, pp 317–350.

D'Amato E, Forlino A, Valli M, Coviello D, Camera G, Ajmar F, Cetta G (1993): SSCP analysis in osteogenesis imperfecta: Detection of a serine for glycine substitution in a patient with osteogenesis imperfecta type IV. Abstract V International Conference on Osteogenesis. Oxford: Imperfecta.

Fedarko N, Moerike M, Brenner R, Gehron Robey P, Vetter U (1992): Extracellular matrix formation by osteoblasts from patients with osteogenesis imperfecta. J Bone Miner Res 7:921–930.

Fisher LW, Termine JD, Young MF (1989): Deduced protein sequence of bone small proteoglycan I (biglycan) shows homology with proteoglycan II (Decorin) and several known non-connective proteins in a variety of species. J Biol Chem 264:4571–4576.

Fleischmajer R, Fisher LW, MacDonald ED, Jacobs L, Perlish JS, Termine JD (1989): Decorin interaction with fibrillar collagen of embryonic and adult human skin. J Struct Biol 106:82–90.

Font B, Aubert-Foucher E, Goldschmidt D, Eichenberger D, van der Rest M (1993): Binding of collagen XIV with the dermatan sulfate side chain of decorin. J Biol Chem 268:25015–25018.

Forlino A, Zolezzi F, Valli M, Pignatti PF, Cetta G, Brunelli PC, Mottes M (1994): Severe (type III) osteogenesis imperfecta due to glycine substitutions in the central domain of the triple helix. Hum Mol Genet 3:2201–2206.

Glössl J, Beck M, Kresse H (1984): Biosynthesis of proteodermatansulfate in cultured human fibroblasts. J Biol Chem 259:14144–14150.

Kresse H, Hausser H, Schönherr E (1993): Small Proteoglycans. Experentia (Basel) 49:403–416.

Mottes M, Sangalli A, Valli M, Gomez-Lira M, Tenni R, Buttita P, Pignatti PF, Cetta (1992): Mild dominant osteogenesis imperfecta with intrafamiliar variability: The cause is a Serine for Glycine α1(I) 901 substitution in type I collagen gene. Hum Genet 89:480–484.

Mottes M, Gomez-Lira M, Valli M, Scarano G, Lonardo F, Forlino A, Cetta G, Pignatti PF (1993): Paternal mosaicism for a COL1A1 dominant mutation (α1 Ser-415) causes recurrent osteogenesis imperfecta. Hum Mut 2:196–204.

Mottes M, Sangalli A, Valli M, Forlino A, Gomez Lira M, Antoniazzi F, Costantinou Deltas CD, Cetta G, Pignatti PF (1994): A base substitution at IVS-19 3′-end splice junction causes exon 20 skipping in pro α2(I) collagen mRNA and produces mild osteogenesis imperfecta. Hum Genet 93:681–687.

Nicholls AC, Oliver J, Renouf DV, Keston M, Pope FM (1991): Substitution of cysteine for glycine at residue 415 of one allele of the α1(I) chain of type I procollagen in type III/IV osteogenesis imperfecta. J Med Genet 28:757–764.

Ponte P, Gunningham P, Blau H, Kedes L (1983): Human actin genes are single copy for α-skeletal and α-cardiac actin but multicopy for β- and γ-cytoskeletal genes: 3′ untranslated regions are isotype specific but conserved in evolution. Mol Cell Biol 3:1783–1791.

Pulkkinen L, Kainulainen K, Kruisius T, Makinen P, Schollin J, Gustavsson KH, Peltonen L (1990): Deficient expression of the gene coding for decorin in a lethal form of Marfan syndrome. J Biol Chem 265:17780–17785.

Quentin E, Gladen A, Roden L, Kresse H (1990): A genetic defect in the biosynthesis of dermatan sulfate proteoglycan galactosyltransferase I deficiency in fibroblasts from a patient with a progeroid syndrome. Proc Natl Acad Sci USA 87:1342–1346.

Raghunath M, Superti-Furga A, Godfrey M, Steinmann B (1993): Decreased extracellular deposition of fibrillin and decorin in neonatal Marfan syndrome fibroblasts. Hum Genet 90:511–515.

Santra M, Skorski T, Calabretta B, Lattime EC, Iozzo R (1995): De novo decorin expression suppresses the malignant phenotype in human colon cancer cells. Proc Natl Acad Sci USA 92:7016–7020.

Schmidt G, Hausser H, Kresse H (1991): Interaction of small proteo*glycan decorin* with fibronectin. Biochem J 280:411–414.

Scott JE (1988): Proteoglycan-fibrillar collagen interactions. Biochem J 252:313–323.

Sztrolovics R, Rimoin DL, Rodriguez E, Roughley PJ (1994): Single-strand conformation polymorphism analysis of human decorin, biglycan and fibromodulin DNAs. Matrix Biol 14:307–312.

Valli M, Mottes M, Tenni R, Sangaui A, Gomez-Lira M, Rossi A, Antoniazzi F, Cetta G, Pignatti PF (1991): A de novo G to T transversion in a pro α1(I) collagen gene for a moderate case of osteogenesis imperfecta. J Biol Chem 266:1872–1878.

Valli M, Sangalli A, Rossi A, Mottes M, Forlino A, Tenni R, Pignatti PF, Cetta G (1993a): Osteogenesis imperfecta and type I collagen mutation: A lethal variant caused by a Gly910Ala substitution in the α1(I). Eur J Biochem 217:77–82.

Valli M, Rossi A, Forlino A, Tenni R, Cetta G (1993b): Extracellular matrix deposition in cultured dermal fibroblasts from four probands affected by osteogenesis imperfecta. Matrix 13:275–280.

Valli M, Zolezzi F, Mottes M, Antoniazzi F, Stanzial F, Tenni R, Pignatti PF, Cetta G (1993c): Gly 85 to val substitution in pro α1(I) chain causes mild osteogenesis imperfecta and introduces a susceptibility to protease digestion. Eur J Biochem 217:77–82.

Vetter U, Fisher LW, Mintz KP, Kopp JB, Tuross N, Termine JD, Gehron Robey P (1991): Osteogenesis imperfecta: Changes in non-collagenous proteins in bone. J Bone Min Res 6:501–505.

Vogel KG, Paulsson M, Heinegard D (1984): Specific inhibition of types I and II collagen fibrillogenesis by the small proteoglycan of tendon. Biochem J 223:587–597.

Vogel KG, Clark P (1989): Small proteoglycan synthesis by skin fibroblasts cultured from elderly donors and patients with defined defects in types I and III collagen metabolism. Eur J Cell Biol 49:236–243.

Winnemöller M, Schmid G, Kresse H (1991): Influence of decorin on fibroblast adhesion to fibronectin. Eur J Cell Biol 54:10–17.

Yamaguchi Y, Mann DM, Ruoslahti E (1990): Negative regulation of transforming growth factor-β by the proteoglycan decorin. Nature 346:281–284.